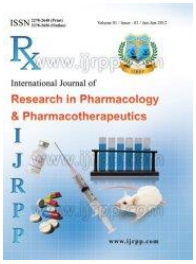
International Journal of Research in Pharmacology & Pharmacotherapeutics



ISSN Print: 2278-2648 IJRPP |Vol.7 | Issue 1 | Jan - Mar - 2018
ISSN Online: 2278-2656 Journal Home page: www.ijrpp.com

Research article Open Access

Influence of *Toddalia asiatica* on cardio biomarkers and its protective role against Isoproterenol Induced Myocardial Infarction in Rats

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ABSTRACT

The objective of the study was to evaluate the influence of ethanolic leaf extract of *Toddalia asiatica* on cardio biomarkers against isoproterenol induced myocardial infarction in rats. The protective activity of *Toddalia asiatica* was assessed by estimating the cardiac marker enzymes (Creatinine Phosphokinase and Lactate Dehydrogenase) and antioxidants enzymes (superoxide dismutase and Catalase). Thirty male Sprague – Dawley rats were randomly allocated in 5 groups of 6 each. Group I served as control, II to V animals were Isoproterenol (85mg/kg) induced myocardiaol infracted animals, pre treated with the reference control Lipistat (350mg/kg) and ethanolic leaf extract of *Toddalia asiatica* (200 & 400 mg/kg). All the test drugs were administered orally for 15 days. On 16th day the blood was collected and subjected to the estimation of cardiac marker enzymes. The animals were sacrificed; hearts were homogenized and used for the estimation of antioxidant enzymes. The result showed that, *Toddalia asiatica* significantly decreased the cardiac marker enzyme and increased the antioxidant enzymes. *Toddalia asiatica* produced a dose dependent cardioprotective effect against the acute cardiac damage induced by Isoproterenol in rats. From the result it was concluded that, *Toddalia asiatica* produced cardioprotective activity against isoproterenol induced cardiac damage in rats which was mediated by its antioxidant property.

Keywords: Isoproterenol, Myocardial Infarction, *Toddalia asiatica*, Cardio Biomarkers and Antioxidants.

INTRODUCTION

Heart diseases, especially coronary artery diseases (CAD), are the leading causes of morbidity and mortality in developed countries. Effective therapy is available to ensure patient survival and to prevent long term sequelae after an acute ischemic event caused by CAD, but appropriate therapy requires rapid and accurate diagnosis. Research into the pathology of CAD have demonstrated the usefulness of measuring concentrations of chemicals released from the injured cardiac muscle can aid the diagnosis of diseases caused by myocardial ischemia. Recently successful better biochemical markers have been described in research publications and applied for the clinical diagnosis of acute ischemic myocardial injury. Aspartate aminotransferase of the 1950s was replaced by other cytosolic enzymes such as lactate dehydrogenase, creatine kinase and their isoenzymes that exhibited better cardiac specificity [1] (Talwar and Srivastava, 2003). Muscle tissue may be damaged following intense prolonged training as a consequence of both metabolic and mechanical factors. Serum levels of skeletal muscle enzymes or proteins are markers of the functional status of muscle tissue, and vary widely in both pathological and physiological conditions. Creatine kinase, lactate dehydrogenase, aldolase, myoglobin, troponin, aspartate aminotransferase, and carbonic anhydrase CAIII are the most useful serum markers of muscle injury [2](Brancaccio *et al*., 2010).

Herbal medicines have been widely utilized as effective remedies for the prevention and treatment of multiple health conditions for centuries by almost every known culture. The first documented records of herbal medicine use date back 5,000 years in China. Similarly, India's Ayurvedic medicine tradition is thought to be more than 5,000 years old and herbal medicines remain an essential component of its practice [3] (Garodia *et al*., 2007). Today, the populations of certain countries still depend on herbal medicines to address their healthcare needs as the modern medicine results with adverse effects and may interfere with the metabolic enzymes leads to other severe complications.

Toddalia asiatica (Lopez root): Rutaceae, a woody liana, is used traditionally in the treatment of various disease conditions. The plant is well known for its antipyretic property. All parts of the plant have characteristic pungent taste. It is used in sprains, contusions, intercostal neuralgia, cough, malaria, dysentery, gastralgia, poisonous snake bites and furuncle infections. Fresh bark of the root is used in the treatment of hill fever. The root is used as dental analgesic. It is also used in odontalgia, paralysis, intermittent fevers, dyspepsia, colic, flatulence, bronchitis, nausea, wounds, filthy ulcers, epilepsy, gonorrhoea, constitutional debility, convalescence after febrile and exhausting diseases, blood motions and arthritis. The root bark is bitter, astringent, acrid, digestive, carminative, constipating, diaphoretic, expectorant, antibacterial, vulnerary, aromatic, tonic, stimulant, antiperiodic, antidiarrhoeal, antipyretic and diuretic. Fresh leaves are eaten raw for pain in the bowel. The leaves are burnt and the ash is used as tooth powder and in decayed teeth. The flowers are useful as an external application in wasp-stings. The unripe fruit is rubbed down with oil to make a stimulant liniment for arthritis. The fruits are used for cough and throat pain. The roots and leaves are boiled and used orally or inhalation for common cold and cough [4](Kirtikar and Basu, 1987). The extract of the plant reported to possess anti-HIV activity [5] (Tandon and Chhor, 2005), antimicrobial [6] (Kar *et al*., 2005) anti-malarial activity [7] (Nyangulu *et al*., 2007), anti-platelet aggregation activity [8] Ian-Lih Tsai *et al*., 1998), analgesic, anti-inflammatory activity, wound healing activity, spasmolytic activity [9] (Lakshmi *et al*., 2002) and anticancer activity [10] (Iwasaki *et al*., 2006). The plant was also reported to reduce the consumption of oxygen and ventricle work, which might be useful in improving the myocardial function [11] (Ye Kai-he *et al*., 2000). The present study was conducted to evaluate the cardioprotective activity and the role of cardio biomarkers in the protective activity of *Toddalia asiatica.*

MATERIALS AND METHODS

Plant Material

The leaves of *Toddalia asiatica*. were collected from the Kolli Hills with the guidance of tribals. The plant samples were identified and authenticated by the Scientist D, Botanical Survey of India, Southern Regional Center, Agricultural University, Coimbatore. The voucher specimen (BSI/SRC/15/86/2017-18/Sci/01237) had been deposited in the herbarium for future reference.

Preparation of Extract

The leaves were washed with water and shade dried. The leaves were crushed with hand to coarse powder. Course powdered leaves of *Toddalia asiatica* were extracted with 90% ethanol by cold maceration for 7 days. The extracts were then concentrated, dried and stored in desiccators.

Animals

Healthy male Sprague – Dawley (SD) rats weighing between 180 - 220 gm were used for this study. The animals were obtained from animal house, Sri Lakshmi Narayana Institute of Medical Sciences, Puducherry. The animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±2^{o}C and relative humidity of 30 – 70 %. A 12:12 light: day cycle was followed. All animals were allowed to free access to water and fed with standard commercial pelleted rat chaw (M/s. Hindustan Lever Ltd, Mumbai). All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee and were in accordance with the Institutional ethical guidelines.

Pharmacological Activity

Cardioprotective Activity [12] (Prabhu *et al*., 2006)

The rats were divided into 5 groups of 6 animals each. Group I served as normal control administered with 1ml/kg of 0.1% CMC solution for 15 days. Group II served as induced control, administered with isoproterenol (85mg/kg, bw) intraperitoneally twice at an interval of 24 hours on 14th and 15th day. Group III served as reference control, administered orally with Lipistat (350 mg/kg, bw), once daily for 15 days. Group IV & V served as test controls, administered orally with 200 and 400 mg/kg, bw of ethanolic leaf extract of *Toddalia asiatica* respectively. Group III, IV and V along with test drugs, the animals administered with isoproterenol (85mg/kg, bw) as mentioned earlier. All the test drugs were administered orally by suspending in 0.1% CMC solution.

On 16th day blood was collected in heparinized tube by retro orbital sinus puncture, under Pentobarbitol Sodium (45mg/kg, i.p) anaesthesia. The collected blood samples were centrifuged for 10 minutes at 2000 r.p.m. and plasma was separated. The separated plasma was subjected to various biochemical tests like estimation of cardiac biomarkers Creatinine Phosphokinase (CPK) [13] (Henry et al., 1960) and Lactate Dehydrogenase (LDH) [14] (Ellman, 1959).

After blood collection, the animals were sacrificed by excess thiopentone sodium and heart tissue was quickly dissected out and washed in ice cold saline. A weighed quantity of each heart was taken from all the groups and a 30% w/v homogenate was prepared in 0.9% buffered KCl (pH 7.4) for the estimation superoxide dismutase (SOD) [15] (Mishra and Fridovich, 1972) and catalase (CAT) [16] (Clairborne, 1985).

Statistical Analysis

Results were expressed as mean ± SEM. The data were analyzed by using one way analysis of variance (ANOVA) followed by Dunnett's *t* test. P values < 0.05 were considered as significant.

RESULT AND DISCUSSION

Table.No: 1. Effect of ethanolic leaf extract of *Toddalia asiatica* on cardiac biomarkers and antioxidant enzymes against Isoproterenol induced myocardial infarction in rats

Groups	Drug Treatment	Plasma		Cardiac Tissue Homogenate	
		CPK (IU/L)	**LDH** (IU/L)	**SOD** (units/mg of protein)	**CAT** (units/mg of protein)
I	Vehicle Control 0.1% CMC	128.33±6.62	132.80±6.36	36.89±1.53	57.28±2.33
II	Isoproterenol (85 mg /kg)	271.42±8.77	221.33±7.43	17.35±1.17	33.75±2.42
III	Lipistat (350mg/kg) + Isoproterenol (85 mg /kg)	159.32±6.29***	162.54±8.45***	31.47±2.05***	51.74±3.66***
IV	*Toddalia asiatica* (200mg/kg) + Isoproterenol (85 mg /kg)	184.04±7.90***	186.50±6.45***	24.82±1.90*	45.42±2.31**
V	*Toddalia asiatica* (400mg/kg) + Isoproterenol (85 mg /kg)	172.66±8.05***	171.55±7.39***	26.54±2.15**	49.77±3.22***

Values are in mean ± SEM (n=6),
*P<0.05, **P<0.01, ***P<0.001 Vs Isoproterenol Control

Toddalia asiatica is widely recognized as a medicinal plant in Africa, India, China and Japan, and it has been used for the treatment of many diseases. It was reported to decreased ventricle work and consumption of oxygen of acute ischemic myocardium improving the diastolic function of myocardium and cardiac output. The study was conducted to evaluate the role of cardio biomarkers (CPK, LDH) and antioxidants in the protective effect of *Toddalia asiatica* against Isoproterenol induced myocardial infarction in rats and the results were shown on table 1. Isoproterenol is a ß – adrenergic, synthetic catechlomine which induces severe stress in the cardiac muscle leading to Myocardial Infarction. Studies suggest that free radical plays a crucial role in the pathogenesis of Isoproterenol induced Myocardial Infarction. Myocardium contains an abundant concentration of diagnostic marker enzymes like CPK, LDH, once the muscle metabolically damaged, these enzymes released its content into the extra cellular fluid [14] (Ellman, 1959). In the present study, the cardioprotective effect of ethanolic leaf extract of *Toddalia asiatica* was assessed by determining the marker enzymes CPK, LDH and the levels of SOD and catalase in cardiac homogenate. In Isoproterenol alone treated animals, the cardiac enzyme levels (CPK and LDH) were increased and the free radical enzymes (SOD and CAT) were decreased, when compared to the levels of control animals, which confirms the damage of cardiac tissues. Pretreatment of Lipistat, the reference control, significantly reduced (P<0.001) the cardiac marker enzymes CPK and LDH and increased (P<0.001) the levels of SOD and CAT. The animals pretreated with 200 & 400 mg/kg of *Toddalia asiatica* significantly (P<0.001) decreased the cardiac marker enzyme CPK and LDH. *Toddalia asiatica* 200mg/kg moderately enhanced the levels of SOD (P<0.05) and CAT (P<0.01), but 400mg/kg of *Toddalia asiatica*, more significantly increased the levels of antioxidant enzymes SOD (P<0.01) and CAT (P<0.001) compared to Isoproterenol control. *Toddalia asiatica* showed dose dependent cardioprotective effect against the acute cardiac damage induced by Isoproterenol in rats by reversing the cardio biomarkers and also due to its antioxidant properties.

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